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Research report

Differential effects of ibogaine on local cerebral glucose utilization in drug-naïve and morphine-dependent rats

Beth Levant*, Thomas L. Pazdernik

Department of Pharmacology, Toxicology, and Therapeutics, University of Kansas Medical Center, Kansas City, KS 66160-7417, USA

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Abstract

Ibogaine, a hallucinogenic indole alkaloid, has been proposed as a treatment for addiction to opioids and other drugs of abuse. The mechanism for its putative anti-addictive effects is unknown. In this study, the effects of ibogaine on local cerebral glucose utilization (LCGU) were determined in freely moving, drug-naïve, or morphine-dependent adult, male, Sprague–Dawley rats using the [^{14}C]2-deoxyglucose (2-DG) method. Morphine-dependent rats were treated with increasing doses of morphine (5–25 mg/kg, s.c., b.i.d.) and then maintained at 25 mg/kg (bid) for 4–7 days. For the 2-DG procedure, rats were injected with saline or ibogaine (40 mg/kg, i.p.). 2-DG was administered 1 h after administration of ibogaine. The rate of LCGU was determined by quantitative autoradiography in 46 brain regions. In drug-naïve animals, ibogaine produced significant increases in LCGU in the parietal, cingulate, and occipital cortices and cerebellum compared to controls consistent with its activity as a hallucinogen and a tremorogen. Morphine-dependent rats had only minor alterations in LCGU at the time assessed in this experiment. However, in morphine-dependent animals, ibogaine produced a global decrease in LCGU that was greatest in brain regions such as the lateral and medial preoptic areas, nucleus of the diagonal band, nucleus accumbens shell, inferior colliculus, locus coeruleus, and flocculus compared to morphine-dependent animals treated with saline. These findings indicate that ibogaine produces distinctly different effects on LCGU in drug-naïve and morphine-dependent rats. This suggests that different mechanisms may underlie ibogaine's hallucinogenic and anti-addictive effects.

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1. Introduction

Ibogaine is an indole alkaloid derived from the root bark of *Tabernanthe iboga*, a shrub native to equatorial Africa [41]. Used for ritual and medicinal purposes, ibogaine produces increased energy, arousal, and appetite at low doses [43]. At higher doses, the drug produces CNS stimulation, tremor, and psychedelic effects such as visual hallucinations, synesthesia, and a “waking dream state” [32,36,42]. Interest in the drug has been stimulated by anecdotal reports suggesting that opioid-dependent

individuals lose the craving for opioids following the use of ibogaine and do not undergo withdrawal [2,29,33,46,47]. This loss of craving has been reported to persist for months to years and appears to occur in persons addicted to a variety of drugs of abuse including cocaine, alcohol, and nicotine, as well as opioids [30,31]. In rats, ibogaine decreases morphine self-administration [10,12,16], an effect that persists for 1 or more days after administration [12,16]. Ibogaine also attenuates naloxone-precipitated withdrawal symptoms in morphine-dependent rats [11,15].

The mechanism of action of ibogaine is complex. The drug binds with micromolar affinity to a variety of sites including the μ and κ opioid receptors, NMDA channel, serotonin and dopamine transporters, σ -1 and 2 sites, voltage-gated sodium channels, 5-HT₂ and 5-HT₃ receptors, and M₁ and M₂ receptors (for review, see

* Corresponding author. Department of Pharmacology, University of Kansas Medical Center, Mail Stop 1018, 3901 Rainbow Boulevard, Kansas City, KS 66160-7417, USA. Tel: +1-913-588-7527; fax: +1-913-588-7501.

E-mail address: blevant@kumc.edu (B. Levant).

Refs. [13,45]). In keeping with these diverse sites of action, ibogaine has effects on a variety of neural systems including the dopaminergic, opioid, serotonergic, cholinergic, GABAergic, glutamatergic, and adrenergic systems (for review, see Refs. [14,43]). The neurochemical basis for ibogaine's putative anti-addictive effects remains to be elucidated.

With the aim of identifying neuronal circuits involved in ibogaine-mediated effects and potential mechanisms of action for the drug's anti-addictive effects, this study investigated the acute effects of a putatively anti-addictive dose of ibogaine on regional neuronal activity in drug-naïve and morphine-dependent rats on the basis of local cerebral glucose utilization (LCGU). We will show that ibogaine produces distinctly different effects on LCGU in drug-naïve and morphine-dependent animals, suggesting different mechanisms for its acute effects in drug-naïve individuals and its putative anti-addictive effects.

2. Materials and methods

All experiments were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals and were approved by the University of Kansas Medical Center Animal Care and Use Committee.

Adult, male, Sprague–Dawley rats (200–220 g; Harlan, Indianapolis, IN) were housed three per cage in a temperature- and humidity-controlled animal facility with a 12-h dark–light cycle (on at 0600 h) and food and water ad libitum. Rats were obtained at least 5 days prior to the commencement of any treatments and were handled daily during the acclimatization period.

2.1. Experimental design

The effects of ibogaine in drug-naïve and morphine-dependent rats were determined in two separate experiments. To assess the effects of ibogaine in a drug-naïve state, rats were treated with a single injection of ibogaine HCl (40 mg/kg, i.p., Sigma, St. Louis, MO; $n=9$) in dH₂O in a volume of 4 ml/kg. This dose of ibogaine has been shown to decrease morphine self-administration and symptoms of withdrawal in rats [10–12,15,16]. Deionized water was used as the drug vehicle because of the limited solubility of ibogaine in saline. Control animals were treated with saline (4 ml/kg; $n=9$). LCGU was determined for the 45-min period beginning 60 min after the administration of ibogaine. The 60-min pretreatment interval was based on the reported effects of ibogaine on locomotor activity and

dopamine release, which are maximal 60 min after administration [17,40].

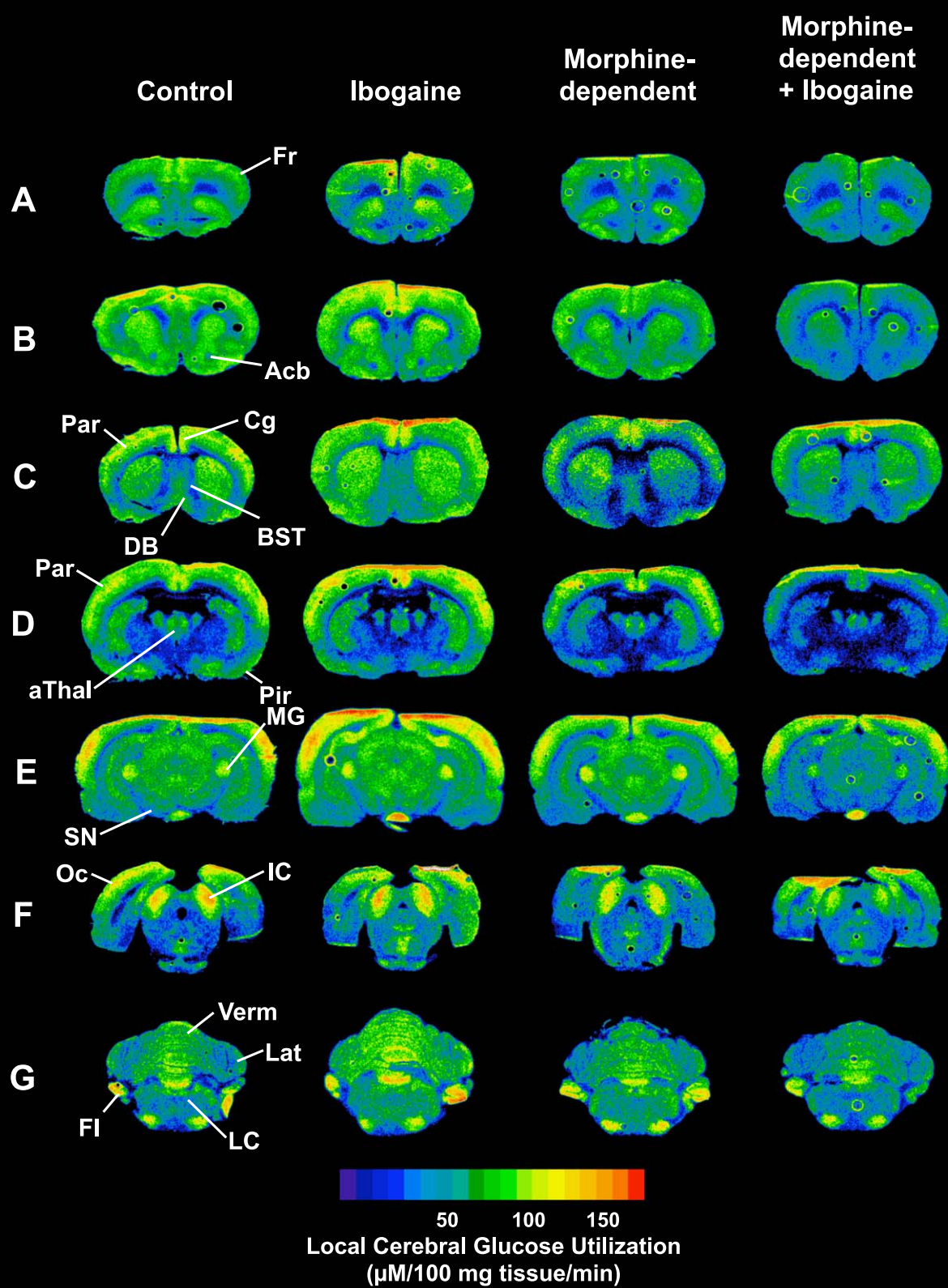
To assess the effects of ibogaine in the morphine-dependent state, rats were randomly assigned to one of three groups: control ($n=7$), morphine-dependent ($n=9$), and morphine-dependent + ibogaine ($n=10$). Morphine-dependent rats were treated with morphine sulfate injection (Baxter, Cherry Hill, NJ) in an increasing dose regimen. The initial morphine dose was 5 mg/kg (b.i.d., s.c.) in a volume of 1 ml/kg. The dose was increased by 5 mg/kg each day until a dose of 25 mg/kg (b.i.d., in a volume of 1.67 ml/kg) was reached. Rats were then maintained at 25 mg/kg (s.c., b.i.d.) for 4–7 days, due to limitations in experimental throughput, before the administration of ibogaine. This treatment protocol produces significant physiological dependence [56]. Control rats were injected with the same volume of saline (b.i.d.). Morphine-dependent rats were assigned to the ibogaine or saline treatment groups for the 2-DG procedure in a stratified manner according to the duration of morphine treatment. On the day of the 2-deoxyglucose (2-DG) procedure, rats were treated with a single injection of ibogaine (40 mg/kg, i.p.) or saline, 12 h after the last injection of morphine. LCGU was determined for the 45-min period beginning 60 min after the administration of ibogaine.

In both experiments, the behavior of the animals was observed during the 2-DG procedure and qualitatively described.

2.2. 2-Deoxyglucose procedure

LCGU was determined using the procedure of Sokoloff et al. [50] in freely moving rats as previously described [5]. Because it is not possible to perform the 2-DG procedure in a large number of animals simultaneously, the 2-DG procedure was performed in a paired and randomized manner until all animals for each experiment were processed. On the day before the 2-DG procedure, the femoral artery and vein were cannulated under pentobarbital anesthesia (50 mg/kg, ip; ~2-h duration). Rats were allowed to recover overnight. LCGU was determined for the 45-min period beginning 60 min after the administration of ibogaine. Fifteen minutes before the administration of 2-DG, 150 U of heparin were injected (i.v.) into each animal and control arterial blood samples were collected in heparinized tubes. [¹⁴C]2-Deoxyglucose (100 μ Ci/kg; 55 Ci/mmol; American Radiolabeled Chemicals, St. Louis, MO) was then administered as a pulse in 0.9% saline via the venous cannula, which was immediately flushed with saline. During the first minute immediately following the pulse, six timed arterial

Fig. 1. Effects of ibogaine on local cerebral glucose utilization (LCGU) in drug-naïve and morphine-dependent rats. Rats were injected with ibogaine (40 mg/kg, i.p.) or saline 60 min prior to the 2-deoxyglucose procedure. (A) Bregma 3.10 mm. (B) Bregma 1.70 mm. (C) Bregma 0.20 mm. (D) Bregma –1.30 mm. (E) Bregma –5.30 mm. (F) Bregma –8.50 mm. (G) Bregma –10.0 mm. Abbreviations—Fr: frontal cortex; Acb: nucleus accumbens; Cg: cingulate cortex; BST: bed nucleus of the stria terminalis; DB: nucleus of the diagonal band; Par: parietal cortex; aThal: anterior thalamus; Pir: piriform cortex; MG: medial geniculate; SN: substantia nigra; Oc: occipital cortex; IC: inferior colliculus; Verm: vermis; Lat: lateral lobe; Fl: flocculus; LC: locus coeruleus.



blood samples (50–70 μ l) were collected. Blood samples were then taken every 5 min. A total of 24 arterial blood samples were collected. Rats were decapitated 45 min after the pulse. Brains were rapidly removed, frozen in isopentane, and stored at -70°C .

Coronal brain sections (20 μ m) were cut on a cryostat, collected on glass cover slips (three sections per cover slip), and immediately dried on a slide warmer. Slide-mounted brain sections were then apposed to Kodak Biomax MR film for 4 days with [^{14}C]methylmethacrylate radioactivity standards. Films were developed according to the manufacturer's instructions. Autoradiographic images were digitized and quantified using the Macintosh-based video densitometry program NIH Image version 1.6. Best fit curves of optical densities generated by the radioactivity standards resulted when a 4 $^{\circ}$ polynomial was used to describe the relationship between optical density and radioactivity. Forty-six brain regions, representing a survey of the fore-, mid-, and hindbrain, were identified according to the atlas of Paxinos and Watson [38]. Sections were sampled bilaterally and averaged for the three sections on each cover slip. Larger brain regions were sampled at multiple levels and the readings averaged to yield one value. The averaged readings for each brain region from each animal, along with plasma glucose levels and [^{14}C] concentrations, were used to calculate the rate of LCGU according to the Sokoloff equation [50]. LCGU in specific brain areas is expressed as micromoles of glucose use per 100 mg tissue per minute. Average cerebral glucose utilization (CGU) was calculated as the average LCGU over all brain regions sampled for each animal as an index of total brain glucose utilization.

2.3. Data analysis

Results are presented as the mean $\pm$ S.E.M. Data were analyzed for statistically significant effects by ANOVA and the Student's *t*-test or Student–Newman–Keuls test, as appropriate. Significance was set at $P < 0.05$.

Table 1

Effects of ibogaine on average cerebral glucose utilization (CGU) in drug-naive and morphine-dependent rats

Group	Average cerebral glucose utilization	
	$\mu\text{mol}/100\text{ mg tissue/min}$	<i>n</i>
<i>Drug-naive study</i>		
Control	74 ± 4.6	(9)
Ibogaine	82 ± 3.7	(9)
<i>Morphine-dependent study</i>		
Control	71 ± 2.8	(7)
Morphine-dependent	71 ± 4.4	(9)
Morphine-dependent + ibogaine	$60 \pm 2.0^{*,\dagger}$	(10)

Rats were injected with ibogaine (40 mg/kg, i.p.) or saline 60 min prior to the 2-deoxyglucose procedure. Data represent the mean $\pm$ S.E.M. and were analyzed by ANOVA and the Student's *t* or Student–Newman–Keuls tests.

* $P < 0.05$ vs. control.

$\dagger$ $P < 0.05$ vs. morphine-dependent.

Table 2

Effects of ibogaine on local cerebral glucose utilization (LCGU) in specific brain regions in drug-naive rats

Brain region/treatment	Local cerebral glucose utilization ($\mu\text{mol}/100\text{ mg tissue/min}$)	
	Control	Ibogaine
Cortex		
Frontal	86 ± 6.4	94 ± 7.9
Infralimbic	76 ± 5.6	84 ± 7.3
Insular	76 ± 6.6	76 ± 6.5
Cingulate	110 ± 7.5	$145 \pm 9.5^{*}$
Parietal	89 ± 7.6	$114 \pm 5.1^{*}$
Piriform	71 ± 5.3	71 ± 4.6
Temporal	129 ± 8.4	138 ± 5.7
Occipital	103 ± 6.1	$139 \pm 6.1^{**}$
Entorhinal	51 ± 3.6	56 ± 4.3
Caudate-putamen	101 ± 7.0	104 ± 7.3
Nucleus accumbens		
Core	88 ± 6.7	85 ± 7.3
Shell	81 ± 5.1	77 ± 6.0
Olfactory tubercle	85 ± 5.5	75 ± 5.9
Septal area	51 ± 4.4	53 ± 5.7
Ventral pallidum	54 ± 4.9	52 ± 5.0
Nucleus of the diagonal band	53 ± 5.1	54 ± 4.7
Bed nucleus of the stria terminalis	48 ± 3.7	$34 \pm 4.3^{*}$
Medial preoptic area	42 ± 4.5	43 ± 2.4
Lateral preoptic area	63 ± 4.4	60 ± 5.4
Globus pallidus	47 ± 4.7	43 ± 5.3
Thalamus		
Anterior	74 ± 6.5	69 ± 6.0
Dorsolateral	114 ± 3.7	122 ± 10.8
Mediolateral	95 ± 4.3	93 ± 6.5
Ventrolateral	109 ± 9.3	115 ± 10.2
Ventromedial	104 ± 10	114 ± 10.8
Hypothalamus		
Anterior	43 ± 4.4	40 ± 3.4
Lateral	52 ± 4.7	50 ± 4.6
Ventromedial	50 ± 3.4	44 ± 3.8
Lateral habenula	96 ± 9.1	115 ± 11.7
Subthalamic nucleus	61 ± 5.3	69 ± 11.0
Amygdala	48 ± 3.6	45 ± 3.7
Dentate gyrus	65 ± 6.2	67 ± 6.5
Hippocampus	62 ± 3.5	59 ± 3.5
Substantia nigra		
Pars compacta	68 ± 4.3	67 ± 3.7
Pars reticulata	53 ± 3.6	52 ± 3.2
Ventral tegmental area	60 ± 4.2	60 ± 4.0
Medial geniculate	121 ± 6.9	126 ± 4.4
Superior colliculus	81 ± 4.5	97 ± 7.6
Inferior colliculus	149 ± 11	162 ± 10.4
Central gray area	66 ± 3.3	82 ± 7.3
Locus coeruleus	58 ± 6.1	68 ± 8.4
Nucleus raphe magnus	45 ± 4.0	49 ± 6.3
Superior olive	100 ± 11	114 ± 10.2
Cerebellum		
Vermis	72 ± 6.4	$106 \pm 9.3^{*}$
Lateral lobes	54 ± 4.6	$81 \pm 9.5^{*}$
Flocculus	100 ± 9.1	$130 \pm 9.2^{*}$

Rats were injected with ibogaine (40 mg/kg, i.p.) or saline 60 min prior to the 2-deoxyglucose procedure ($n = 9$ rats per group). Data represent the mean $\pm$ S.E.M. and were analyzed by ANOVA and the Student's *t*-test.

* $P < 0.05$.

** $P < 0.01$.

Table 3
Effects of ibogaine on local cerebral glucose utilization (LCGU) in specific brain regions in morphine-dependent rats

Brain region/ treatment	Local cerebral glucose utilization ($\mu\text{mol}/100 \text{ mg tissue}/\text{min}$)		
	Control	Morphine- dependent	Morphine- dependent + ibogaine
Cortex			
Frontal	86 $\pm$ 4.2	78 $\pm$ 6.5	76 $\pm$ 2.4
Infralimbic	71 $\pm$ 4.9	70 $\pm$ 6.0	63 $\pm$ 3.0
Insular	71 $\pm$ 3.7	69 $\pm$ 6.4	58 $\pm$ 1.8
Cingulate	121 $\pm$ 5.0	109 $\pm$ 8.7	115 $\pm$ 6.3
Parietal	96 $\pm$ 4.0	88 $\pm$ 6.8	80 $\pm$ 2.5
Piriform	71 $\pm$ 4.1	66 $\pm$ 5.4	56 $\pm$ 2.2*
Temporal	122 $\pm$ 8.2	110 $\pm$ 8.8	99 $\pm$ 5.5
Occipital	100 $\pm$ 7.0	87 $\pm$ 7.5	82 $\pm$ 4.2
Entorhinal	46 $\pm$ 3.6	44 $\pm$ 3.8	41 $\pm$ 3.4
Caudate-putamen	88 $\pm$ 3.6	88 $\pm$ 6.5	78 $\pm$ 3.2
Nucleus accumbens			
Core	67 $\pm$ 3.9	66 $\pm$ 5.5	57 $\pm$ 2.5
Shell	65 $\pm$ 6.1	69 $\pm$ 5.9	51 $\pm$ 2.3
Olfactory tubercle	71 $\pm$ 7.5	71 $\pm$ 5.9	56 $\pm$ 3.0
Septal area	45 $\pm$ 3.4	50 $\pm$ 4.2	41 $\pm$ 2.5
Ventral pallidum	47 $\pm$ 2.5	51 $\pm$ 5.0	39 $\pm$ 2.7
Nucleus of the diagonal band	62 $\pm$ 3.3	71 $\pm$ 5.7	48 $\pm$ 3.6* ^{††}
Bed nucleus of the stria terminalis	32 $\pm$ 3.2	37 $\pm$ 3.3	29 $\pm$ 2.4
Medial preoptic area	48 $\pm$ 6.4	56 $\pm$ 7.7	36 $\pm$ 2.5 [†]
Lateral preoptic area	56 $\pm$ 4.1	70 $\pm$ 6.3*	45 $\pm$ 2.8 ^{††}
Globus pallidus	44 $\pm$ 3.5	45 $\pm$ 4.1	38 $\pm$ 3.0
Thalamus			
Anterior	78 $\pm$ 5.1	78 $\pm$ 6.7	58 $\pm$ 4.2* [†]
Dorsolateral	114 $\pm$ 5.0	99 $\pm$ 7.8	94 $\pm$ 2.9
Mediolateral	96 $\pm$ 4.0	91 $\pm$ 7.6	79 $\pm$ 2.9
Ventrolateral	97 $\pm$ 4.0	90 $\pm$ 6.8	82 $\pm$ 2.8
Ventromedial	96 $\pm$ 4.2	90 $\pm$ 6.7	79 $\pm$ 4.4
Hypothalamus			
Anterior	38 $\pm$ 3.4	42 $\pm$ 3.9	33 $\pm$ 3.1
Lateral	52 $\pm$ 3.0	58 $\pm$ 5.8	47 $\pm$ 4.6
Ventromedial	42 $\pm$ 2.7	42 $\pm$ 2.6	37 $\pm$ 2.4
Lateral habenula	89 $\pm$ 5.6	98 $\pm$ 5.5	83 $\pm$ 7.1
Subthalamic nucleus	48 $\pm$ 3.2	47 $\pm$ 3.2	44 $\pm$ 3.0
Amygdala	44 $\pm$ 2.7	42 $\pm$ 3.1	38 $\pm$ 2.4
Dentate gyrus	53 $\pm$ 3.1	49 $\pm$ 3.1	47 $\pm$ 2.8
Hippocampus	56 $\pm$ 3.3	54 $\pm$ 3.6	49 $\pm$ 2.9
Substantia nigra			
Pars compacta	59 $\pm$ 3.9	59 $\pm$ 4.9	46 $\pm$ 4.2
Pars reticulata	44 $\pm$ 3.1	44 $\pm$ 4.4	39 $\pm$ 3.7
Ventral tegmental area	51 $\pm$ 4.3	55 $\pm$ 4.7	41 $\pm$ 4.1
Medial geniculate	108 $\pm$ 7.2	111 $\pm$ 7.8	92 $\pm$ 7.0
Superior colliculus	73 $\pm$ 6.7	71 $\pm$ 5.4	55 $\pm$ 4.0
Inferior colliculus	171 $\pm$ 4.9	145 $\pm$ 12*	113 $\pm$ 5.6*** ^{††}
Central gray area	53 $\pm$ 5.5	53 $\pm$ 4.4	43 $\pm$ 3.6
Locus coeruleus	53 $\pm$ 3.0	54 $\pm$ 2.3	43 $\pm$ 3.2* [†]
Nucleus raphe magnus	38 $\pm$ 3.4	38 $\pm$ 2.3	29 $\pm$ 2.5
Superior olive	110 $\pm$ 8.9	121 $\pm$ 9.1	104 $\pm$ 9.1
Cerebellum			
Vermis	69 $\pm$ 4.1	73 $\pm$ 6.2	65 $\pm$ 2.9
Lateral lobes	49 $\pm$ 2.8	50 $\pm$ 3.7	47 $\pm$ 2.7
Flocculus	111 $\pm$ 8.4	107 $\pm$ 8.4	86 $\pm$ 4.4* [†]

3. Results

The acute effects of ibogaine on LCGU were determined in drug-naïve and morphine-dependent rats. LCGU was measured for a 45-min period beginning 60 min after ibogaine administration. Control LCGU values were not significantly different between the drug-naïve and morphine-dependent experiments. Representative autoradiograms are presented in Fig. 1.

Drug-naïve rats injected with ibogaine (40 mg/kg, i.p.) exhibited overt behavioral effects including decreased locomotor activity, piloerection, and tremor, consistent with previous reports [12,34]. Average CGU in ibogaine-treated animals was not significantly different from saline-treated, drug-naïve animals (control) (Table 1). Specific brain regions with significant increases in LCGU compared to control included several cortical areas including cingulate (+32%), parietal (+28%), and occipital (+35%) cortices. Ibogaine also produced significant increases in LCGU in the cerebellar vermis (+47%), lateral lobes (+51%), and flocculus (+30%), whereas a significant decrease was observed in the bed nucleus of the stria terminalis (−29%) (Table 2).

LCGU was assessed in saline-treated, morphine-dependent rats 12 h after the last injection of morphine. At this time, rats exhibited no symptoms of overt withdrawal such as wet-dog shakes, diarrhea, lacrimation, rhinorrhea, etc. Average CGU in morphine-dependent animals was not significantly different from control at the time assessed in this experiment (Table 1). Analysis of specific brain regions revealed a significant increase in LCGU in the lateral preoptic area (+25%) and a decrease in LCGU in the inferior colliculus (−15%) compared to control (Table 3).

The overt behavioral effects of ibogaine in morphine-dependent animals were similar to those observed in drug-naïve animals. However, in distinct contrast to the effects of ibogaine in drug-naïve animals, ibogaine produced a decrease in average CGU in morphine-dependent animals compared to control (−16%) or morphine-dependent animals (−16%) (Table 1). Likewise, significant decreases in LCGU compared to morphine-dependent animals were observed in the lateral and medial preoptic areas (−36% and −35%, respectively), nucleus of the diagonal band (−33%), nucleus accumbens shell (−26%), anterior thalamus (−25%), inferior colliculus (−23%), locus coeruleus (−21%), and the flocculus (−20%). When compared to controls, significant

Notes to Table 3:

Rats were injected with ibogaine (40 mg/kg, i.p.) or saline 60 min prior to the 2-deoxyglucose procedure. The sample size was 7 rats per group for control, 9 for morphine-dependent, and 10 for morphine-dependent + ibogaine. Data were analyzed by ANOVA and the Student–Newman–Keuls test.

* $P < 0.05$.

*** $P < 0.001$ vs. control.

† $P < 0.05$.

†† $P < 0.01$ vs. morphine-dependent.

decreases were also observed in nucleus of the diagonal band (–24%), anterior thalamus (–25%), inferior colliculus (–34%), locus coeruleus (–19%), flocculus (–23%), and piriform cortex (–21%), but not in the nucleus accumbens shell or the medial and lateral preoptic areas (Table 3).

4. Discussion

The effects of a putatively anti-addictive dose of ibogaine on LCGU were determined in drug-naïve and morphine-dependent rats using the [^{14}C]2-deoxyglucose method. This method measures the accumulation of a radiolabeled glucose analogue in neurons, which correlates with neuronal activity [49]. As measured in this study, LCGU represents the net uptake and phosphorylation of glucose for the neuronal population sampled in a given brain region. Because 2-deoxyglucose-6-phosphate does not enter the Krebs cycle, this method does not assess any subsequent steps in glucose metabolism. LCGU was determined 1 h after administration of ibogaine. At that time point, both ibogaine and its active metabolite, noribogaine, are present in brain [39]. Thus, the effects on LCGU reported here represent the net effect of the actions of these two compounds.

4.1. Effects of ibogaine in drug-naïve animals

Used at a dose shown to decrease morphine self-administration and symptoms of withdrawal in rats [10–12,15,16], ibogaine produced significant increases in LCGU in the cingulate, parietal, and occipital cortices in drug-naïve animals. The increases in LCGU in these cortical brain regions are similar to the increases in cerebral blood flow in the frontal and parietal cortices observed after lysergic acid diethylamide (LSD) administration in conscious rats [18]. Likewise, increased glucose uptake is observed in cortical brain regions in humans treated with psilocybin, which correlates with visual hallucinations and disintegration of thought processes [52]. In another study, however, LSD and several other indolamine and piperazine 5-HT agonists decreased LCGU in variety of brain regions including many cortical areas [20]. This discrepancy between the findings of Grome and Harper [20] and those of the present study and Goldman et al. [18] may be due to the use of different drugs and doses, the use of restrained or freely moving animals, and/or differences in time allowed for recovery from anesthesia between the studies. Thus, the increased LCGU in cortical brain regions produced by ibogaine appear to be generally similar to the effects of other hallucinogens.

The similarity between the effects of ibogaine and other hallucinogens on indices of neuronal activity also concurs with observations that the subjective effects of these drugs are similar in humans and in discriminative stimulus tests

in animals [22,32,36,42]. Taken together, these findings suggest that the dose of ibogaine used in this study is hallucinogenic. They also suggest that the hallucinogenic effects of the drugs are mediated by similar mechanisms. The mechanisms of action of LSD, psilocybin, and other hallucinogens are not well understood but involve multiple actions on the 5-HT system, particularly activation of 5-HT_{2A} receptors [1]. Ibogaine binds to the 5-HT transporter with low micromolar affinity and appears to increase extracellular levels of 5-HT by either blocking uptake and/or releasing the neurotransmitter [55]. Activation of the 5-HT_{2A} receptor, either directly or as a result of increased synaptic availability of 5-HT, appears to be an important component of the ibogaine's discriminative stimulus properties [21,23]. Thus, on the basis on ibogaine's effects on LCGU, actions on the serotonergic system appear to play an important role in the drug's acute hallucinogenic effects.

Ibogaine-induced alterations in LCGU in subcortical brain areas are also consistent with the drug's acute pharmacological effects. Increased LCGU in the cerebellum suggests activation of motor systems associated with the induction of tremor and concurs with the effects of the tremorogen harmaline on LCGU in cats [3]. Decreased LCGU in the bed nucleus of the stria terminalis suggests effects on the serotonergic limbic circuitry connecting the amygdala and the hypothalamus [54].

Whereas the acute effects of ibogaine on LCGU are concordant with its hallucinogenic and tremorogenic activity, the pattern of alterations in LCGU is not consistent with several proposed mechanisms for the drug's anti-addictive effects, specifically activity as a noncompetitive antagonist at the *N*-methyl-D-aspartate (NMDA) glutamate receptor subtype or as a *mu* opioid agonist. Ibogaine has relatively high affinity in competition with the NMDA antagonist [^3H]MK-801 and blocks the effects of glutamate in several functional assays (for review, see Ref. [48]). However, the effects of acute treatment with ibogaine on LCGU differ from the pattern of alterations produced by MK-801 in rats. For example, although both ibogaine and MK-801 increase LCGU in the cingulate cortex, ibogaine increases LCGU in the occipital cortex whereas LCGU in that region was decreased by MK-801 [7,28]. MK-801 also increases LCGU in thalamic regions, hippocampus, and substantia nigra, brain regions in which LCGU was not altered by ibogaine [28]. Likewise, it has been postulated that ibogaine blocks opioid withdrawal through the *mu* agonist activity of its metabolite noribogaine [37]. However, the acute effects of ibogaine on LCGU are also dissimilar from those produced by acute treatment with morphine, which decreases LCGU in many of brain areas including the basal ganglia, thalamus, and hippocampus [4,8]. Thus, the effects of ibogaine on LCGU do not support a predominant action of the drug as a noncompetitive NMDA antagonist or a *mu* agonist in vivo in drug-naïve animals.

4.2. Effects of morphine dependence on LCGU

Whereas acute treatment with morphine produces widespread decreases in LCGU [4,8], naloxone-induced morphine withdrawal increases LCGU in many brain regions [26,57], although decreases in LCGU occur in some brain regions [57]. These studies [26,57], however, differ in their findings on the effects of chronic morphine treatment. Similar to Kimes and London [26], who found no significant alterations in LCGU in morphine-dependent rats, we observed altered LCGU in only 2 of 46 brain regions. This very limited effect on LCGU suggests the development of tolerance and physiological dependence and supports the observation that the rats were not experiencing withdrawal symptoms at the time of ibogaine administration.

4.3. Effects of ibogaine on LCGU in morphine-dependent animals

Acute administration of ibogaine in morphine-dependent rats produced a distinctly different pattern of alterations in LCGU than that observed in drug-naïve rats. Whereas drug-naïve rats exhibited increases in LCGU in specific brain regions associated with hallucinogenic and tremorogenic activity but no change in average CGU after ibogaine treatment, morphine-dependent rats given ibogaine exhibited a significant decrease in average CGU compared to either drug-naïve or morphine-dependent rats. Moreover, in morphine-dependent animals, ibogaine produced significant decreases in LCGU compared to control or morphine-dependent groups in a variety of brain regions associated with the mesolimbic dopaminergic, adrenergic, auditory, and other systems. The observation of effects in these brain regions is consistent with previous studies of ibogaine's pharmacological effects suggesting actions on a variety of neural systems (for review, see Refs. [14,43]).

Although the magnitude of the decrease in LCGU did not reach statistical significance in a number of brain regions, it is noteworthy that ibogaine decreased LCGU in morphine-dependent rats relative to control in all brain regions examined and all but one brain area compared to the morphine-dependent group. Although LCGU in cingulate cortex of the ibogaine-treated, morphine-dependent animals was slightly higher (+6%) than in morphine-dependent animals, LCGU in this brain area was increased 32% by ibogaine in drug-naïve animals. Thus, in morphine-dependent animals, the effects of ibogaine on LCGU appear to be more widespread than in drug-naïve animals and result in a global decrease in LCGU.

It has been hypothesized that ibogaine produces its anti-addictive effects and blocks opioid withdrawal by mitigating or reversing the neuroadaptations underlying physiological dependence and/or addiction [32]. The decrease in average CGU produced by ibogaine in morphine-dependent rats is the opposite of the widespread increases in LCGU reported

in rats undergoing withdrawal [26,57] and might thus counteract or prevent some of the symptoms of withdrawal. Likewise, drug craving produced by opioids and other addictive substances is associated with increased neuronal activity in limbic brain areas such as the limbic cortex and nucleus accumbens [6,9,19,25,51]. Similarly, sensitization to morphine, which is hypothesized to contribute to addiction and craving, also produces increases in LCGU in the nucleus accumbens and limbic cortex in rats [27]. Accordingly, the reported loss of craving in opioid addicts treated with ibogaine could result from the prevention or reversal of increased neuronal activity in these brain areas.

The mechanism by which ibogaine decreases average CGU in morphine-dependent animals remains to be determined. The effects of ibogaine on LCGU in morphine-dependent animals appear similar to the acute effects of morphine in drug-naïve animals [4,8] and could reflect a reversal of the neuroadaptations underlying tolerance and/or addiction. Such a reversal of neuroadaptations could occur at the level of intracellular signaling cascades. Of note, although ibogaine has no effect alone on adenylyl cyclase activity, it has been shown to potentiate the inhibition of adenylyl cyclase produced by a maximally effective concentration of morphine [44]. Alternatively, ibogaine given to morphine-dependent rats might produce global effects on neuronal energy utilization, through mechanisms such as altered insulin sensitivity [53] or modulation of neural sensors that regulate global cerebral metabolic activity [24,35], and thus heterologously counteract neuroadaptations associated with addiction.

5. Conclusion

The present data demonstrate that a putatively anti-addictive dose of ibogaine produces distinctly different effects on LCGU in drug-naïve and morphine-dependent rats. This suggests that different mechanisms underlie ibogaine's hallucinogenic and anti-addictive effects and is consistent with hypotheses that ibogaine may counteract neuroadaptations underlying addiction. Future studies must elucidate the precise mechanism(s) of action of this drug. Finally, the dramatic differences between the effects of ibogaine on LCGU in drug-naïve and morphine-dependent rats strongly suggest that there are important differences between the nondependent and the opioid-dependent brain and that the anti-addictive mechanism of ibogaine may be best elucidated in drug-dependent, rather than drug-naïve, animals.

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